

NREL-Amoco CRADA Phase 3

Bench Scale Report 2.1

Continuous Fermentation of Corn Fiber Hydrolyzate by LNH33C

Project Title: Amoco-NREL CRADA with corn fiber

Researcher: John Carpenter @ 384-6146

Director of Research: George Philippidis @ 384-6888

Date of experiment: August 10, 1995

Date of Report: August 28, 1995

NREL Technical Record Book: No. 1646, p. 101

Objective

To investigate the fermentation performance of the yeast LNH33C on corn fiber hydrolyzate.

Materials and Methods

Yeast strain

The organism used in these studies was the genetically engineered Purdue recombinant yeast LNH33C. The seed vials were prepared by growing the cells in YEPX media for 18 hours, then diluting 1/2 with a 40% (w/v) glycerol solution and quick freezing. The organism originated at Purdue University (Dr. Nancy Ho) and was supplied to NREL by Amoco Corporation.

Inoculum Preparation

A 1-mL frozen vial stored at -70° C was thawed at room temperature and inoculated into 50 mL YEPX with 2% (w/w) xylose and 1% (w/w) CSL media. The first stage inoculum was incubated at 30° C in a rotary shaker (150 rpm) for 30 hours. The second stage inoculum was started with a 10% inoculum from the first stage. The media composition was the same as the first stage and was grown for 18 hours.

Growth Media

The corn fiber media was prepared by adding 2% (w/w) corn steep liquor (GPC), and 1% (w/w) yeast extract to 15 liters of pretreated corn fiber supernatant. The corn fiber was pretreated using a proprietary Amoco process. The hydrolyzate (pH 1.6) was autoclaved at 121° C for 90 minutes.

Inoculation

The fermenter was charged with 900 mL of the hydrolyzate media described above and the pH was adjusted to 4.91 with 52 mL of 5M NaOH. The fermenter was then inoculated with 100 mL of the second stage inoculum.

Growth Conditions

The continuous fermentation temperature was maintained at 30° C and the agitation speed at 150 r.p.m. The steady state volume was 1000mL with a resident time of 72 hours.

Analytical techniques

Glucose and ethanol concentrations were determined using a Hewlett Packard 1090 HPLC equipped with a 1047 IR detector, HPX-87XH, and a HPX-87XP column. Column temperature was 85° C. Samples were centrifuged and sterile filtered (0.2µ).

Results and Discussion.

The fermentation experienced a considerable lag of over 24 hours compared to cultures grown in ideal media (media without the inhibitors present in hydrolyzate), which usually have the glucose utilized within 20 hours. The glucose was gradually metabolized ~~from 20~~ g/L to 0.5 g/L in about 40 hours during the batch phase of fermentation. After ~~the~~ start of the continuous phase at 47 hours of the fermentation, the glucose level increased and remained steady at around 2.7 g/L, implying a continuous production of glucose from oligomers or ~~an~~ inability of the yeast to metabolize the glucose in that residence time or both. The optical density remained steady throughout the fermentation ~~at 15~~ (@600nm). The viability also remained steady at 3×10^7 CPU/mL and the plasmid stability ~~at 90%~~. In this fermentation, the xylose ~~is not being~~ co-fermented ~~and~~ remains unchanged at 30 g/L, which may be the result of inhibition by the 5.5 g/L acetic acid present in the hydrolyzate and other unidentified inhibitors. Another explanation for the remaining glucose and xylose ~~is~~ that the residence time is not long enough for the organism to utilize these sugars. The yields have not been calculated at this time due to the lack of data on the oligomeric forms of glucose and xylose present in the fermentation vessel. Possible solutions to the lack of xylose utilization include increasing the residence ~~time~~, increasing the pH to diminish the effects of acetic acid, removing the inhibitors in the hydrolyzate, providing continuous inoculum, adjusting the nutrients to optimal levels, increasing cell numbers, and increasing aeration.

Conclusions

Although the organism ~~was~~ capable of fermenting glucose in the hydrolyzate, it experienced a long lag phase ~~and~~ was unable to utilize any xylose at the residence time of 72 hours. All fermentation indicators such ~~as~~ glucose concentration, optical density, CFUs, and ethanol concentration remained steady throughout the run indicating a steady state fermentation.

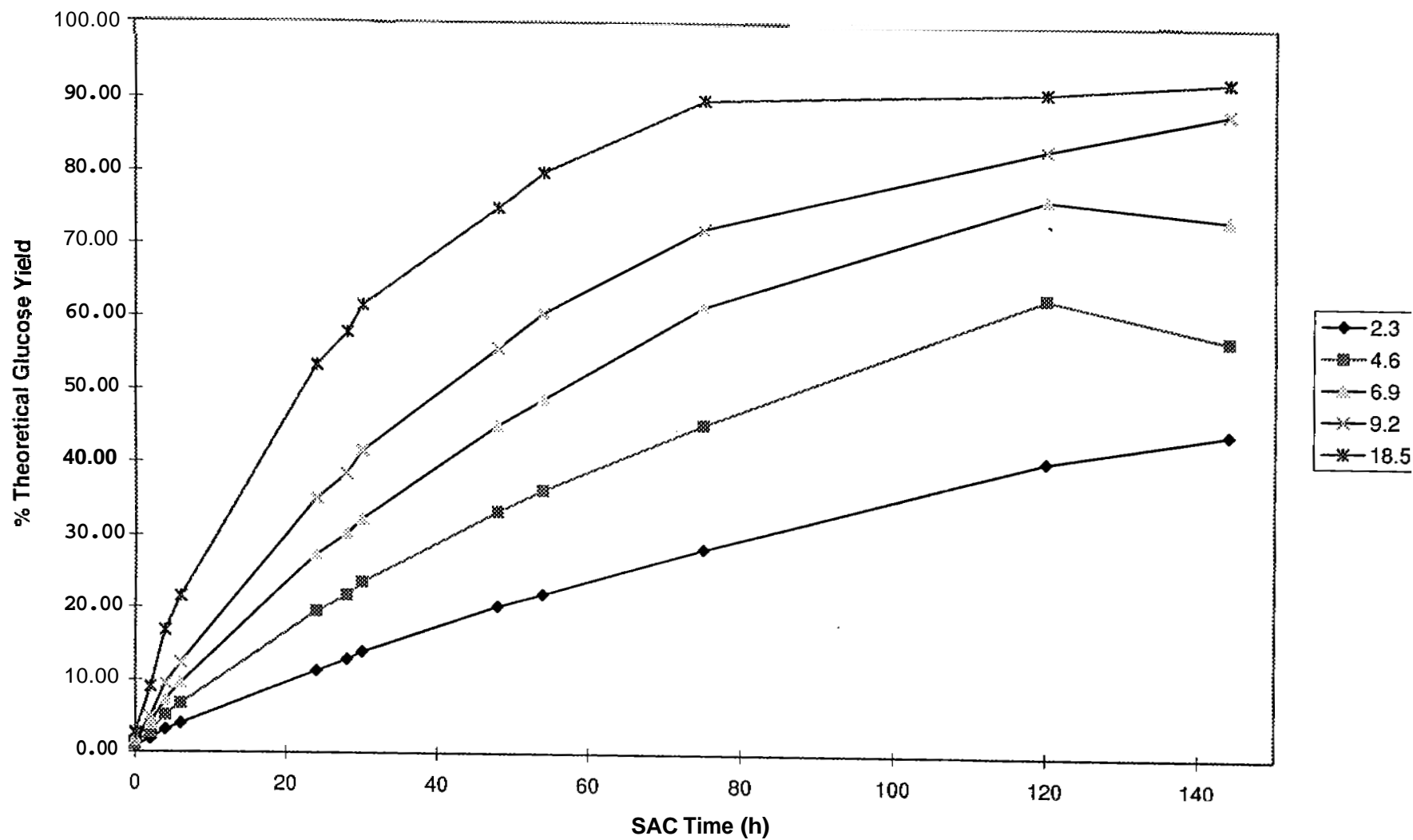
Experiment	CAT Analysis #95-088																			
	1		2		3		4		5		6		7		8		9		10	
Time (h)	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose
0	0.58	0.33	0.58	0.33	0.65	0.51	0.65	0.51	1.08	0.61	1.08	0.61	1.33	0.44	1.33	0.44	2.46	0.68	2.46	0.68
2	0.63	0.75	0.7	0.2	1.07	1.09	1.11	1	1.62	1.36	1.81	1.28	2.12	1.51	2.35	1.25	3.99	2.2	5.42	1.25
4	1.01	1.07	1.02	1.04	1.44	1.76	1.71	1.4	2.53	1.86	2.47	1.88	3.3	2.32	3.2	2.26	6.32	2.82	6.81	2.45
6	1.16	1.19	1.3	1.25	2.08	1.74	2.1	1.67	2.97	2.3	2.93	2.22	4.09	2.63	3.96	2.6	7.6	3.36	7.32	3.5
24	3.06	2.21	3.25	2.29	5.63	3.3	5.4	3.12	7.89	3.97	6.07	4.06	10.20	4.51	9.42	4.23	15.14	4.39	15.87	4.52
28	3.51	2.37	3.71	2.51	6.28	3.34	5.98	3.3	8.64	4.12	8.92	4.22	11.16	4.55	10.34	4.25	16.81	4.37	16.19	4.35

Experiment 2		CAT Analysis #95-088														
	1		2		3		4		5		6		7		8	
Time (h)	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose	Glucose	Cellobiose
0	1.33	0.64	1.33	0.61	1.79	0.77	1.94	0.72	2.43	1	3.23	0.92	3.97	1.26	5.31	0.73
3	2.69	1.99	2.77	2.01	4.11	2.61	4.64	1.89	6.72	3.42	6.42	3.57	10.22	4.11	10.25	3.93
5	3.75	2.54	3.79	2.59	5.72	3.02	5.46	2.53	8.46	4.27	8.49	4.31	13.33	4.47	13.69	3.8
8	4.68	2.87	4.19	2.83	6.33	3.68	6.05	3	7.62	3.02	9.86	4.35	15.41	4.4	14.08	4.62
24	9.47	4.01	9.45	4.36	12.74	4.5	12.91	4.97	17.64	4.33	17.49	4.52	22.43	3.36	22.01	3.32
31	10.94	4.4	11.39	4.18	15.37	3.91	16.7	5.7	18.92	3.96	19.72	3.97	24.1	2.82	23.37	2.78
49	14.47	4.62	14.39	4.6	18.15	4.09	19.44	4.86	27.3	3.77	22.53	2.97	25.73	1.84	30.89	2.38
72	17.35	4.09	18.03	3.95	20.99	3.18	31.45	5.53	25.05	1.68	24.58	1.93	26.85	1.35	29.33	1.54

	5%, 10		10%, 5		10%, 10		10%, 15 IU		10%, 20	
SSF Time	1	2	3	4	5	6	7	8	9	10
0	1.18	1.18	1.18	1.18	1.18	1.18	1.18	1.18	1.18	1.18
24	7.28	7.22			10.6	9.86	13.5	12.06	18.3	17.82
56	11.95	12	12.4	13.7	23.45	22.2	27.85	26.55	28.9	29.35
78	12.8	13.1	16.6	19.15	28.3	24.96	26	25.95	26.65	31.4
96	12.1	13.45	19.5	18.8	26.3	25.7	31.4	25.3	31.25	30.85
122			23.5	22.7						
167	13.2	15.75	23.65	23.05	27	28.3	28.4	23.5	28.35	28.6

YSI Glucose measured after enzyme **denatured**.

Saccharification of Washed Corn Fiber



Saccharifications of Corn Fiber

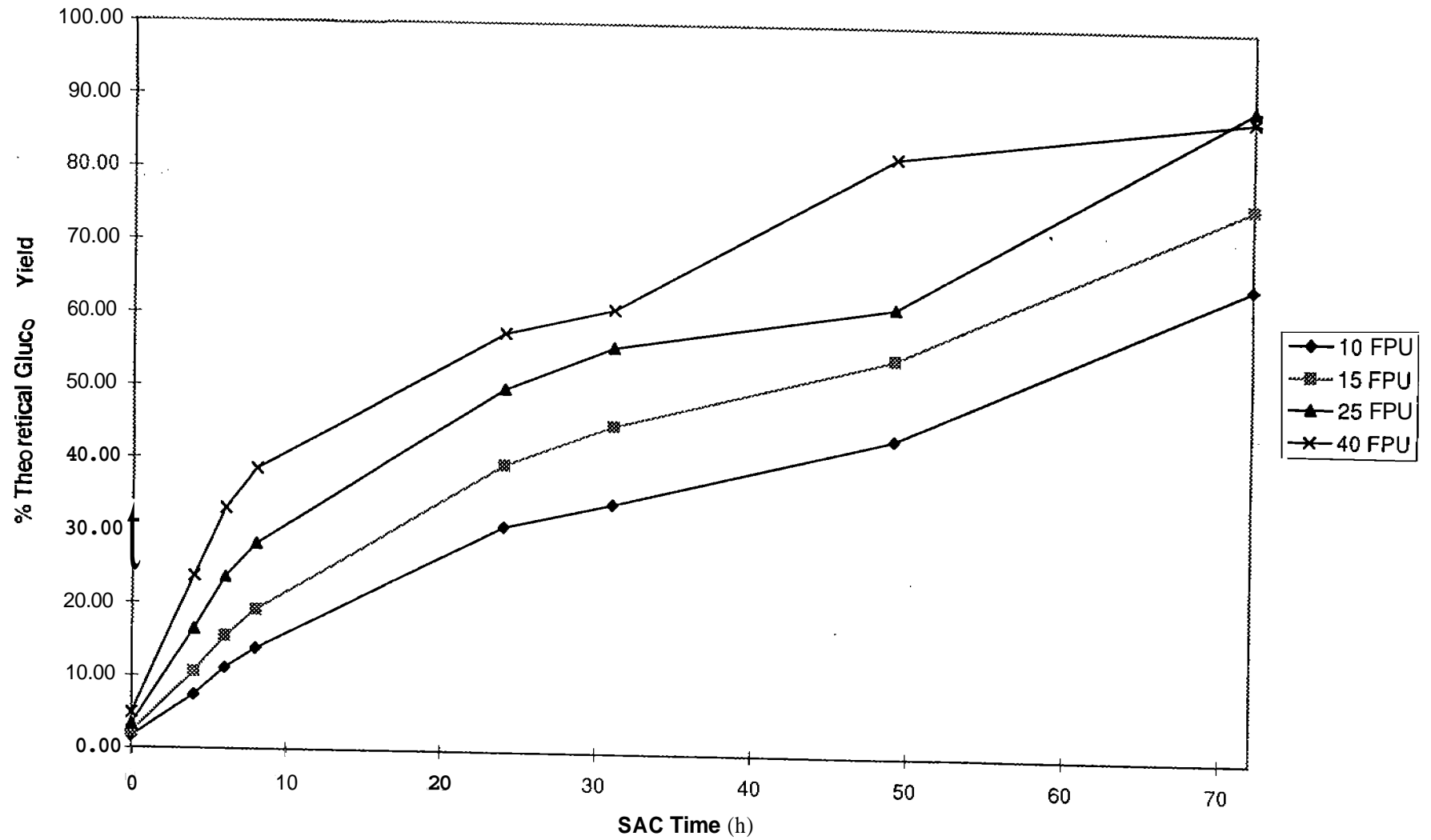
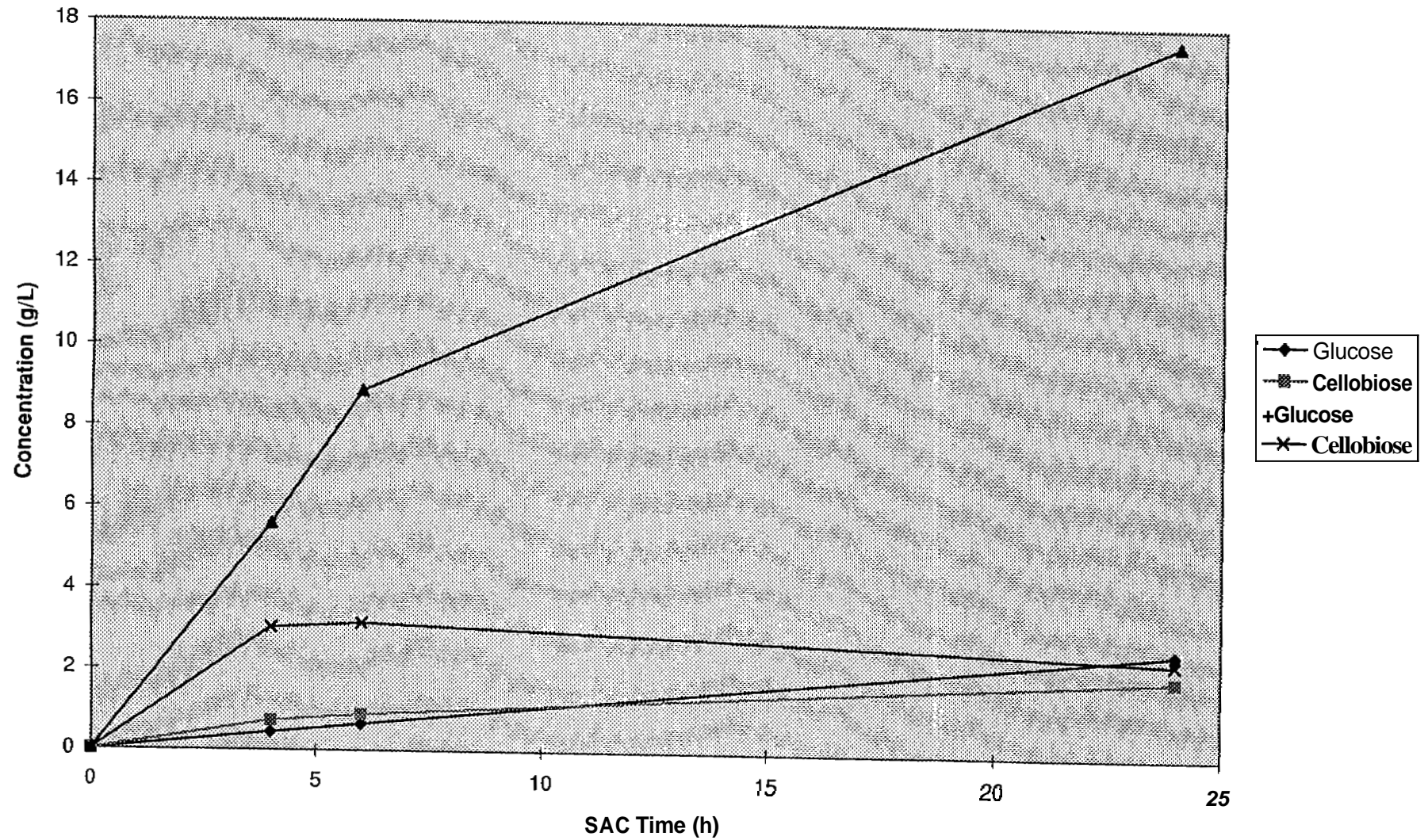


Chart1

Glucose and Cellobiose Levels



Total Sugars Released

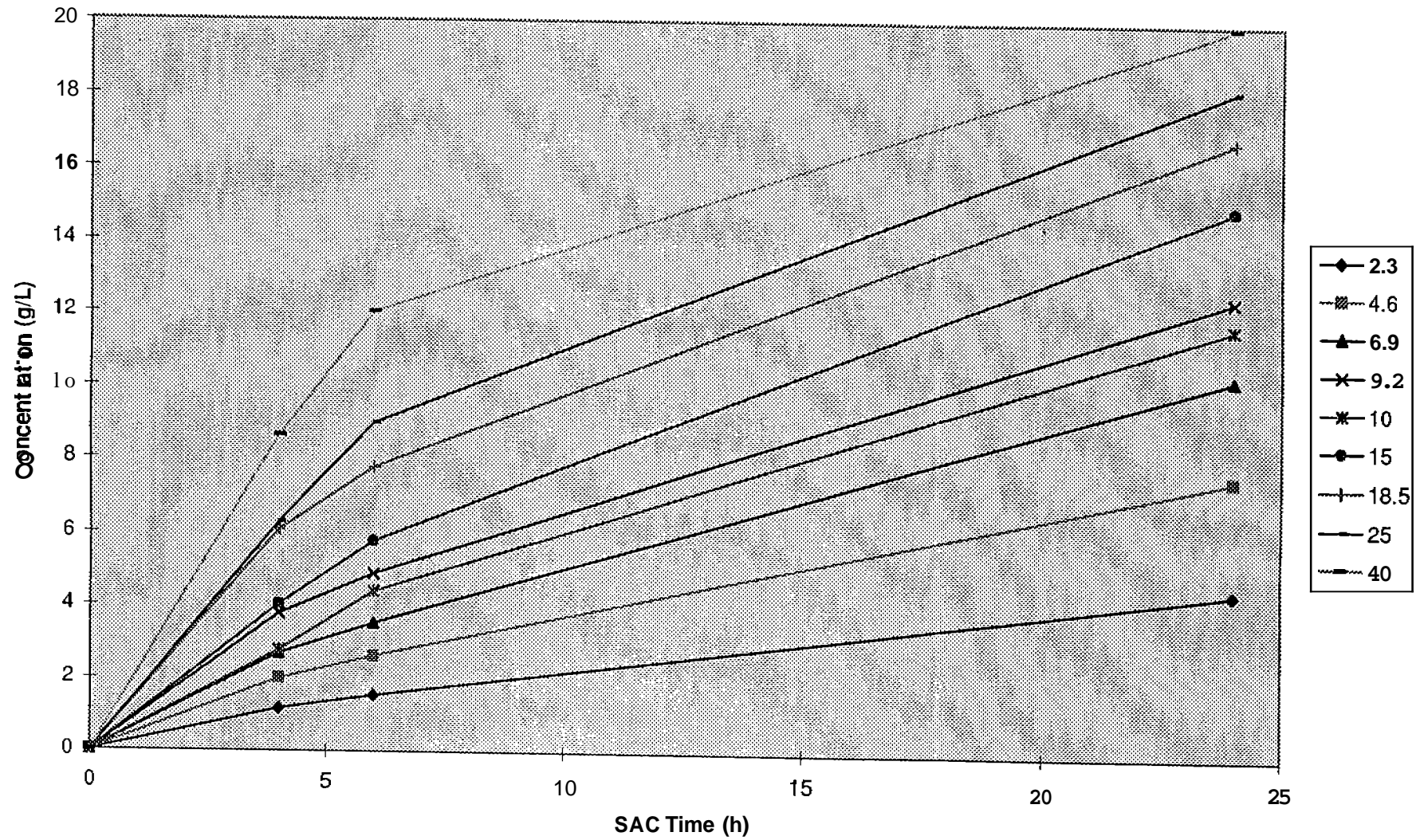
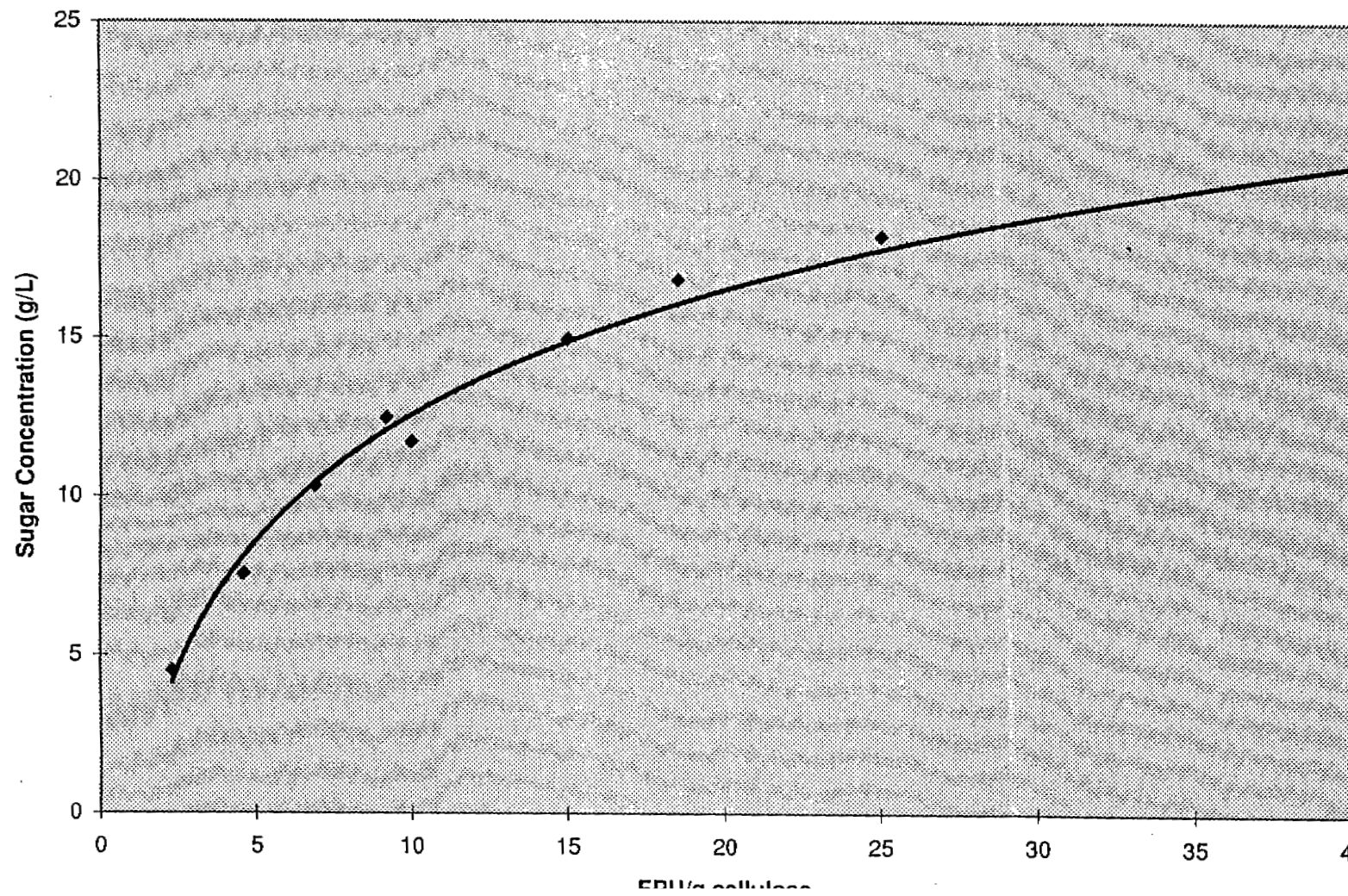
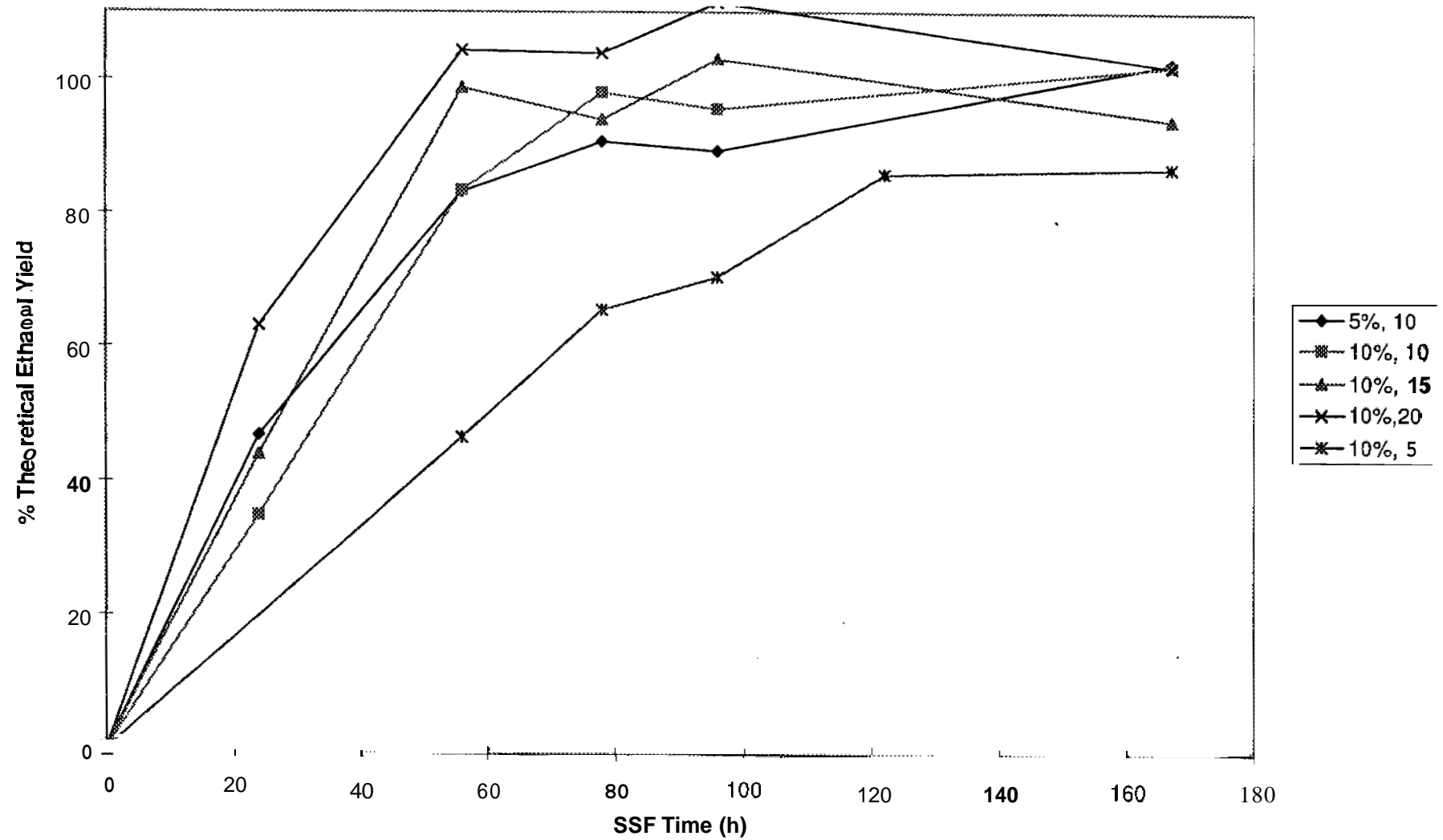


Chart4

Relationship between Enzyme Loading and Sugar Released



Enzyme Loading on Washed ECF SSFs



CHEMICAL ANALYSIS & TESTING

(CAT) Task Analytical Report

Analysis
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Project Title: AMOCO CRADA, Enzyme Loading - ECF, Saccharification I and II (ET60)

NREL In-House ☐Current Subcontractor ☐CRADA ☐Other ☐

Name of Project Contact Person: Tammy Kay Hayward

Date Work Completed: 7/13/95

NREL Notebook: #1561, p046-047

Date Samples Delivered: 6/15/95

Sample Description: Saccharification I and II timepoints

Actual Hours Spent: 38

Summary of Requested Work: Cellobiose and glucose present in samples as received.

Proposed Approach: Cellobiose and glucose analyzed in house by HPX-87H column chromatography, not run in duplicate.

Work Required: ☐ Sample Prep ☐ Acid Digest ☐ HPLC ☐ YSI ☐ GC ☐ Other:

Results and Comments ☐ % As Received ☐ % Dry Weight ☐ mg/mL ☐ Other:

	glu	0.58	0.69	1.01	1.16	3.06	3.51							
2	Saccharification I. Enzyme Loading - Corn Fiber 2	cel	0.33*	0.82	1.04	1.25	2.29	2.51						
	glu	0.58*	0.70	1.02	1.30	3.25	3.71							
3	Saccharification I. Enzyme Loading - Corn Fiber 3	cel	0.51	1.09	1.44	1.74	3.30	3.34						
	glu	0.65	1.07	1.76	2.08	5.63	6.18							
4	Saccharification I. Enzyme Loading - Corn Fiber 4	cel	0.51*	1.00	1.40	1.67	3.12	3.30						
	glu	0.65*	1.11	1.71	2.10	5.40	5.98							
5	Saccharification I. Enzyme Loading - Corn Fiber 5	cel	0.61	1.36	1.86	2.30	3.97	4.12						
	glu	1.08	1.62	2.53	2.97	7.89	8.64							
6	Saccharification I, Enzyme Loading - Corn Fiber 6	cel	0.61*	1.25	1.88	2.22	4.06	4.22						
	glu	1.08*	1.81	2.47	1.93	8.07	8.92							

Name(s) of CAT Staff Working on Project: Larry Brown,
Ray Ruiz

Reviewed by: Tina Ehman

CC: Christos Hatzis

NREL CRADA Protected Information

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Results and Comments

☐

% As Received

☐

% Dry Weight

☐

mg/mL

☐

Other:

Sample (95-088-621), cont.

t=0

2 hr

4 hr

6 hr

24 hr

28 hr

7	Saccharification I, Enzyme Loading - Corn Fiber 7	cel	0.44	1.51	2.32	2.63	4.51	4.55						
		glu	1.33	2.12	3.30	4.09	10.18	11.16						
8	Saccharification I. Enzyme Loading - Corn Fiber 8	cel	0.44*	1.25	2.26	2.60	4.23	4.25						
		glu	1.33*	2.35	3.10	3.96	9.47	10.34						
9	Saccharification I. Enzyme Loading - Corn Fiber 9	cel	0.68	2.20	2.82	3.36	4.39	4.37						
		glu	2.46	3.99	6.32	7.60	15.14	16.81						
10	Saccharification I. Enzyme Loading - Corn Fiber 10	cel	0.68*	1.25	2.45	3.50	4.52	4.35						
		glu	2.46*	5.42	6.81	7.32	15.87	16.19						

☐

Sample (95-088-622)

t=0

3 hr

5 hr

8 hr

24 hr

31 hr

49 hr

72 hr

1	Saccharification II. Enzyme Loading - Corn Fiber 1	cel	0.64	1.99	2.54	2.87	4.01	4.40	4.62	4.09				
		glu	1.33	2.69	3.72	4.68	9.47	10.94	14.47	17.35				
2	Saccharification II. Enzyme Loading - Corn Fiber 2	cel	0.61	2.01	2.59	2.83	4.36	4.18	4.60	3.95				
		glu	1.33	2.77	3.79	4.19	9.45	11.39	14.39	18.03				
3	Saccharification II. Enzyme Loading - Corn Fiber 3	cel	0.77	2.61	3.02	3.68	4.50	3.91	4.09	3.18				
		glu	1.79	4.11	5.72	6.33	12.74	15.37	18.15	20.99				
4	Saccharification II. Enzyme Loading - Corn Fiber 4	cel	0.72	1.89	2.53	3.00	4.97	5.70	4.86	5.53				
		glu	1.94	4.64	5.46	6.05	12.91	16.70	19.44	31.45				
5	Saccharification II. Enzyme Loading -	cel	1.00	3.42	4.27	3.02	4.33	3.96	3.77	1.68				

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CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

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Results and Comments

☐

% As Received

☐

% Dry Weight

☐

mg/mL

☐

Other:

Sample (95-088-621), cont.

t=0

2 hr

4 hr

6 hr

24 hr

28 hr

7	Saccharification I, Enzyme Loading - Corn Fiber 7	cel	0.44	1.51	2.32	2.63	4.51	4.55						
		glu	1.33	2.12	3.30	4.09	10.18	11.16						
8	Saccharification I. Enzyme Loading - Corn Fiber 8	cel	0.44*	1.25	2.26	2.60	4.23	4.25						
		glu	1.33*	2.35	3.10	3.96	9.47	10.34						
9	Saccharification I. Enzyme Loading - Corn Fiber 9	cel	0.68	2.20	2.82	3.36	4.39	4.37						
		glu	2.46	3.99	6.32	7.60	15.14	16.81						
10	Saccharification I. Enzyme Loading - Corn Fiber 10	cel	0.68*	1.25	2.45	3.50	4.52	4.35						
		glu	2.46*	5.42	6.81	7.32	15.87	16.19						

☐

Sample (95-088-622)

t=0

3 hr

5 hr

8 hr

24 hr

31 hr

49 hr

72 hr

1	Saccharification II. Enzyme Loading - Corn Fiber 1	cel	0.64	1.99	2.54	2.87	4.01	4.40	4.62	4.09				
		glu	1.33	2.69	3.72	4.68	9.47	10.94	14.47	17.35				
2	Saccharification II. Enzyme Loading - Corn Fiber 2	cel	0.61	2.01	2.59	2.83	4.36	4.18	4.60	3.95				
		glu	1.33	2.77	3.79	4.19	9.45	11.39	14.39	18.03				
3	Saccharification II. Enzyme Loading - Corn Fiber 3	cel	0.77	2.61	3.02	3.68	4.50	3.91	4.09	3.18				
		glu	1.79	4.11	5.72	6.33	12.74	15.37	18.15	20.99				
4	Saccharification II. Enzyme Loading - Corn Fiber 4	cel	0.72	1.89	2.53	3.00	4.97	5.70	4.86	5.53				
		glu	1.94	4.64	5.46	6.05	12.91	16.70	19.44	31.45				
5	Saccharification II. Enzyme Loading -	cel	1.00	3.42	4.27	3.02	4.33	3.96	3.77	1.68				

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CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

Analysis
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95-088

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Results and Comments

☐

% As Received

☐

% Dry Weight

☐

mg/mL

☐

Other:

Sample (95-088-621), cont.

t=0

2 hr

4 hr

6 hr

24 hr

28 hr

7	Saccharification I, Enzyme Loading - Corn Fiber 7	cel	0.44	1.51	2.32	2.63	4.51	4.55						
		glu	1.33	2.12	3.30	4.09	10.18	11.16						
8	Saccharification I. Enzyme Loading - Corn Fiber 8	cel	0.44*	1.25	2.26	2.60	4.23	4.25						
		glu	1.33*	2.35	3.10	3.96	9.47	10.34						
9	Saccharification I. Enzyme Loading - Corn Fiber 9	cel	0.68	2.20	2.82	3.36	4.39	4.37						
		glu	2.46	3.99	6.32	7.60	15.14	16.81						
10	Saccharification I. Enzyme Loading - Corn Fiber 10	cel	0.68*	1.25	2.45	3.50	4.52	4.35						
		glu	2.46*	5.42	6.81	7.32	15.87	16.19						

☐

Sample (95-088-622)

t=0

3 hr

5 hr

8 hr

24 hr

31 hr

49 hr

72 hr

1	Saccharification II. Enzyme Loading - Corn Fiber 1	cel	0.64	1.99	2.54	2.87	4.01	4.40	4.62	4.09				
		glu	1.33	2.69	3.72	4.68	9.47	10.94	14.47	17.35				
2	Saccharification II. Enzyme Loading - Corn Fiber 2	cel	0.61	2.01	2.59	2.83	4.36	4.18	4.60	3.95				
		glu	1.33	2.77	3.79	4.19	9.45	11.39	14.39	18.03				
3	Saccharification II. Enzyme Loading - Corn Fiber 3	cel	0.77	2.61	3.02	3.68	4.50	3.91	4.09	3.18				
		glu	1.79	4.11	5.72	6.33	12.74	15.37	18.15	20.99				
4	Saccharification II. Enzyme Loading - Corn Fiber 4	cel	0.72	1.89	2.53	3.00	4.97	5.70	4.86	5.53				
		glu	1.94	4.64	5.46	6.05	12.91	16.70	19.44	31.45				
5	Saccharification II. Enzyme Loading -	cel	1.00	3.42	4.27	3.02	4.33	3.96	3.77	1.68				